Development of a standardized strategy for detection of total and viable protozoan parasites *Cryptosporidium*, *Giardia* and *Toxoplasma* in food matrices

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INTRODUCTION

Giardia intestinalis, *Cryptosporidium* spp and *Toxoplasma gondii* are emerging pathogen parasites which have now to be considered in the food domain[1]. *Giardia* cysts, and *Cryptosporidium* and *Toxoplasma* oocysts are excreted in high quantities in the environment by infected hosts. Their spreading in waters and soils, where they can survive and retain their infectivity for several months, is a major source of contamination for humans and animals. Hence, these protozoa are directly associated to waterborne outbreaks with more than 30 000 cases these last 15 years[2]. Moreover spoiled waters can contaminate vegetables and mollusks during primary production. Then transmission to the consumers can occur by ingestion of raw or undercooked matrices. Despite of this, parasitic foodborne outbreaks remain neglected, the main reason being the absence of standardized method for their detection in food matrices. In this context, the Protofood ANR consortium was set up in order to develop a complete global strategy allowing the extraction, detection, and characterization of *Giardia*, *Cryptosporidium* and *Toxoplasma* (oo)cysts in vegetables. The developed tools were then used to evaluate the effect of storage at refrigerated temperature on protozoa.

GLOBAL STRATEGY FOR DETECTION AND CHARACTERIZATION OF *GIARDIA INTESTINALIS*, *CRYPTOSPORIDIUM* spp AND *TOXOPLASMA GONDII* IN VEGETABLES

ELUTION FROM MATRICES

CONCENTRATION/PURIFICATION OF (OO)CYSTS: IMMUNOMAGNETIC SEPARATION (IMS)

Development of an IMS specific of *Toxoplasma gondii*

Antibody 3G4 reacts with the oocyst wall (sporulated and non sporulated oocysts)

	LIQUID MATRICES	FOOD MATRICES
RECOVERY RATE	53±6%,	1 to 4%
LIMIT OF DETECTION (LOD)		
- Microscopy	50 oocysts/ml	3.3 oocysts/g
- PCR	50 oocysts/ml	0.3 oocyst/g

Conclusions:

☺ Limits of detection are low on two matrices (improved in PCR on food matrice).

☺ IMS *Toxoplasma* is promising for extraction from environmental samples (rate of 53%).

☹ IMS *Toxoplasma* is not suitable for application on food matrices (too low rates).

DETECTION OF PROTOZOA BY REAL-TIME PCR [3,4] (DNA, i.e. viable and dead)

	TARGET GENE	LIMIT OF DETECTION *
G. intestinalis	M54878.1 (16S-like rRNA)	1 (oo)cyst / reaction
C. parvum	AF188110 (DNA J like protein)	
T. gondii	AF487550.1 (529pb RE)	

* LOD = Limit of quantification (LOQ)

DETECTION OF VIABLE PROTOZOA BY REAL-TIME RT-PCR (mRNA)

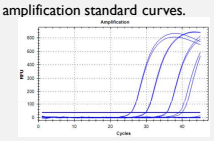
[if positive detection by PCR]

	TARGET GENE	LIMIT OF DETECTION
G. intestinalis	FR751246.1 (beta-giardin)	2 (oo)cyst / reaction
C. parvum	U71181.1 (hsp70)	
T. gondii	AY492338.1 (SporoSAG)	

	RECOVERY RATE	LIMIT OF DETECTION (oo)cysts / g of matrice	
		PCR*	RT-PCR
G. intestinalis	5%	3	3
C. parvum	10%	3	
T. gondii	26%	< 1	

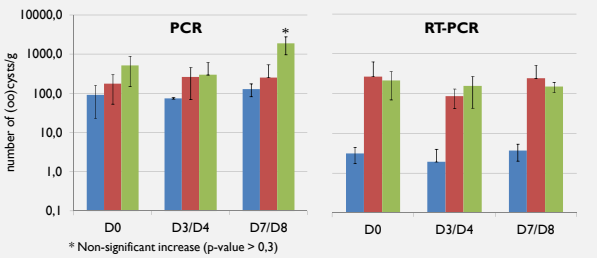
Recovery rate = N/N_0.
N: number of (oo)cysts recovered at the end of extraction.
N_0: number of (oo)cysts artificially inoculated on food matrice.
Protozoa are quantified using genomic DNA amplification standard curves.

*Validated by inter-laboratories assays



IMPACT OF STORAGE AT 4°C ON QUANTITY AND VIABILITY OF (OO)CYSTS

* *T. gondii*: 340 oocysts/g; *C. parvum* & *G. intestinalis*: 4350 (oo)cysts/g.



* Non-significant increase (p-value > 0,3)

Conclusions:

- The number of total protozoa on basil's leaves remains stable during storage at 4°C.
- Viability of protozoa is not affected by storage at 4°C.
- Only 3% of oocysts of *T. gondii* detected by PCR are viable.

CONCLUSION

This project allowed to develop a rapid procedure to extract and detect the protozoa *Giardia intestinalis*, *Cryptosporidium* spp and *Toxoplasma gondii* in vegetable matrices which are considered to be at high risk for human when eaten raw. This detection strategy can be combined to other methods such as real-time RT-PCR to estimate the viability of the protozoa and then to evaluate their infectious potential. Using this procedure, we showed that the protozoa are able to persist at the surface of leaves of vegetables and remain viable during storage at 4°C.
This toolbox is of great interest to better evaluate the risk for humans in foods, especially in terms of occurrence, contamination routes and impact of processes. Moreover, this work represents an added value regarding the international regulation which is now in progress (ISO 18744) .

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AKNOWLEDGMENTS

This work was funded by the French National Research Agency (grant ANR-09-ALIA-009) and the Regional Council of Basse-Normandie.
This study was also supported by the AQUIMER and PEIFL competitiveness clusters.